REMARKS

Amendments to the claims are made for the purpose of presenting the rejected claims in better form for appeal. The amendments do not raise new issues and do not require any further consideration or search by the Examiner. Applicant submits that the amendments overcome all grounds of final rejection and respectfully request their entry.

By this Amendment, claims 1, 2, 4 and 7 are amended, and claims 18-32 are cancelled. Claims 1-7, 9, 10, 12, 14, and 15 are pending in the application.

Claim 1 is amended to incorporate the limitation from claim 18 which specifies that the inflammatory or metabolic disorder is "selected from the group consisting of type 2 diabetes mellitus, metabolic syndrome, and inflammation caused by osteoarthritis." Claims 1, 2, 4 and 7 are amended to replace the term preventing with inhibiting in order to better define the invention. Dependent claims 4 and 7 are amended to cancel subject matter that is not encompassed by independent claim 1, as amended, from which claims 4 and 7 depend. No new matter is added.

Claim 18 is cancelled as its subject matter is made redundant following the amendment to claim 1.

Claims 19-32 drawn to irbesartan are cancelled.

Cancellation of claims 19-32 to irbesartan and amendment of claim 1 to specific inflammatory or metabolic disorders should not be construed as a surrender or dedication to the public of the subject matter removed by these amendments and cancellations. Applicants expressly reserve the right to pursue these claims in a continuation or divisional application.

Applicant is entitled to an advisory action based on the filing of this amendment and response within the two month expedited period. Further, Applicant believes that the present application is in condition for allowance based on the amendments and arguments presented herein. The Examiner is encouraged to contact Applicant's representative regarding any matter that is deemed to expedite the issuance of the claims.

Reconsideration of the application is respectfully requested in view of the above amendments and the following remarks. For the Examiner's convenience, Applicant's remarks are presented in the order in which they were raised in the Office Action.

A. Telephone interview with the Examiner

Applicant expresses his appreciation to Examiner Weddington for discussing the application with Applicant's representative. The Examiner's suggestions for amendments to the claims and identification of allowable subject matter is incorporated in this response.

B. Obviousness-type Double Patenting Rejection

Claims 1-7, 9, 10, 12, 14, 15 and 18-32 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 58-87 of copending Application No. 10/801,437.

Applicant notes that claims 58-87 of copending Application No. 10/801,437 are not in condition for allowance at this time. Applicant will file a terminal disclaimer in the later issued patent, if any, to avoid non-statutory double patenting.

C. Claim Rejections Under 35 U.S.C. §112, first paragraph

Claims 1-7, 9, 10, 12, 14, 15, and 18-31 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. Claims 18-31 are cancelled.

The Examiner contends that the specification, while being enabling for treating type 2-diabetes, metabolic syndrome and inflammation caused by osteoarthritis with telmisartan, does not reasonably provide enablement for treating *all* inflammatory or metabolic disorders or prophylactically preventing an inflammatory or metabolic disorder by administering all compounds, including irbesartan, sufficient to at least partially activate peroxisome proliferators activated receptors (PPARs) and at least partially inhibit, antagonize or block an activity of angiotensin II type 1 receptors.

In response Applicant amends independent claim 1, which is drawn to treatment with "telmisartan," to specify that the inflammatory or metabolic disorder is "selected from the group

consisting of type 2 diabetes mellitus, metabolic syndrome, and inflammation caused by osteoarthritis. The Examiner has acknowledged that the specification is enabling for treating type 2-diabetes, metabolic syndrome and inflammation caused by osteoarthritis with telmisartan. (Office Action, page 4). Claims 2-7, 9, 10, 12, 14, and 15 depend from claim 1.

The Examiner further contends that the specification does not provide any test result that shows that telmisartan does in fact prevent inflammatory or metabolic disorders in healthy mammals that do not have any such inflammatory or metabolic disorders. However, the Examiner notes that results in the Specification do show that mammals that had the disorder or presently have it are protected from recurrence of the disorder by telmisartan.

Applicant presents an article authored by, among others, the inventor of this application, which provides examples of protecting against metabolic problems and diabetes by administering telmisartan to a healthy animal that does not have any inflammatory or metabolic disorder, in accordance with the properties of telmisartan described in the specification and claims. Benson *et al.* "Identification of Telmisartan as a Unique Angiotensin II Receptor Antagonist With Selective PPAR–Modulating Activity." *Hypertension*. 43:993-1002.(2004).A copy of the publication is enclosed for the Examiner's convenience.

In Benson *et al.* healthy male Sprague Dawley rats were placed on a high fat, high carbohydrate diet at 6 weeks of age. The high fat, high carbohydrate diet mimics American diets and administration of this diet for more than 4 weeks can help promote metabolic disturbances leading to diabetes. Within 2 days after starting the diet, the rats were randomized to receive either telmisartan (oral telmisartan; dose = 5 mg/kg body weight per day) or no drug treatment (control). After 5 weeks, serum levels of glucose, insulin, and triglycerides were obtained in the semi-fasting state. *See* pages 994-995 of Benson *et al.*

Serum glucose levels measured after 5 weeks were significantly increased in the control group compared to the telmisartan group ($P<.001$). Thus, the telmisartan protected these healthy animals from developing the increases in serum glucose levels known to result in diabetes. Telmisartan also prevented the increases in serum insulin levels observed in control animals ($P=.025$ by one-tailed t testing). Telmisartan also protected against the increases in serum triglyceride levels seen in the control group ($P<.05$). *See* page 998 and Figure 5 of Benson *et al.*

These findings demonstrate that oral administration of telmisartan protects against the development of metabolic problems and diabetes in healthy animals and the effect is related to its PPAR-γ modulatng activity.

During the telephone interview with the Examiner, the Examiner suggested amending the claim language from "prophylactically preventing" to "prophylactically inhibiting" in order to better reflect the results shown in the specification. Applicant has amended claim 1, 2, 4 and 7 accordingly.

Therefore, Applicant respectfully requests withdrawal of this ground for rejection of claims 1-7, 9, 10, 12, 14, and 15 under 35 U.S.C. § 112, first paragraph, for lack of enablement.

D. Claim Rejections Under 35 U.S.C. § 102(b)

Claims 19-32 stand rejected under 35 U.S.C. § 102(b) as being anticipated by O'Donnell et al.

Claims 19-32 are cancelled. Therefore, the grounds for rejection of these claims are moot.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Applicants request entry of these amendments and request the Examiner to expedite prosecution of this patent application to issuance. Should the Examiner have any questions, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 421842000400. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: August 26, 2005.

Respectfully submitted,

By _____

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Identification of Telmisartan as a Unique Angiotensin II Receptor Antagonist With Selective PPARγ–Modulating Activity

Stephen C. Benson, Harrihar A. Pershadsingh, Christopher I. Ho, Amar Chittiboyina, Prashant Desai, Michal Pravenec, Nianning Qi, Jiaming Wang, Mitchell A. Avery, Theodore W. Kurtz

Abstract—The metabolic syndrome is a common precursor of cardiovascular disease and type 2 diabetes that is characterized by the clustering of insulin resistance, dyslipidemia, and increased blood pressure. In humans, mutations in the peroxisome proliferator–activated receptor-γ (PPARγ) have been reported to cause the full-blown metabolic syndrome, and drugs that activate PPARγ have proven to be effective agents for the prevention and treatment of insulin resistance and type 2 diabetes. Here we report that telmisartan, a structurally unique angiotensin II receptor antagonist used for the treatment of hypertension, can function as a partial agonist of PPARγ; influence the expression of PPARγ target genes involved in carbohydrate and lipid metabolism; and reduce glucose, insulin, and triglyceride levels in rats fed a high-fat, high-carbohydrate diet. None of the other commercially available angiotensin II receptor antagonists appeared to activate PPARγ when tested at concentrations typically achieved in plasma with conventional oral dosing. In contrast to ordinary antihypertensive and antidiabetic agents, molecules that can simultaneously block the angiotensin II receptor and activate PPARγ have the potential to treat both hemodynamic and biochemical features of the metabolic syndrome and could provide unique opportunities for the prevention and treatment of diabetes and cardiovascular disease in high-risk populations. (***Hypertension*. 2004;43:993-1002.)**

Key Words: receptors, angiotensin II ■ angiotensin II ■ renin-angiotensin system ■ insulin resistance ■ losartan

All currently available classes of antihypertensive drugs were developed before it was widely recognized that increased blood pressure is closely associated with insulin resistance and dyslipidemia and well before public health authorities established diagnostic criteria for the metabolic syndrome.[1–3] Thus, the antihypertensive drugs in use today were designed primarily to affect cellular and biochemical mechanisms contributing to increased blood pressure and not to address the disordered carbohydrate and lipid metabolism that often accompany hypertension as part of the metabolic syndrome. Given the major impact of the metabolic syndrome on cardiovascular disease morbidity and mortality,[4–6] the availability of antihypertensive agents that also improve insulin resistance and dyslipidemia could be of considerable clinical value.

Numerous studies have demonstrated that the peroxisome proliferator–activated receptor-γ (PPARγ) plays an important role in regulating carbohydrate and lipid metabolism and that ligands for PPARγ can improve insulin sensitivity, reduce triglyceride levels, and decrease the risk for atherosclerosis.[7–15] PPARγ ligands also have modest antihypertensive effects related at least in part to their ability to promote peripheral vasodilation.[16–19] Several thiazolidinedione ligands for PPARγ have been approved for the treatment of type 2 diabetes; however, these agents have limited capacity to reduce blood pressure and can provoke fluid retention, weight gain, edema, and heart failure in a significant proportion of patients with diabetes.[20–22] Such side effects can also occur with nonthiazolidinedione ligands of PPARγ and are unlikely to be related to the thiazolidinedione moiety per se.[18,23,24] Thus, currently approved synthetic ligands for PPARγ cannot be used to effectively treat the abnormal hemodynamic features of the metabolic syndrome and are associated with adverse effects that are of particular concern for diabetic individuals predisposed to impaired cardiac function.

Recently, we observed an interesting structural resemblance between telmisartan, an angiotensin II (Ang II) type 1 receptor antagonist approved for the treatment of hypertension, and pioglitazone, a PPARγ ligand approved for the treatment of type 2 diabetes.[25] This discovery supported the possibility that certain molecules might have the capacity not

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only to block the Ang II receptor, a key cell surface receptor involved in the regulation of blood pressure,[26] but also to activate PPARγ, an intracellular nuclear hormone receptor involved in the regulation of carbohydrate and lipid metabolism. Theoretically, such bifunctional molecules could treat both the hemodynamic and biochemical features of the metabolic syndrome and have greater potential for preventing atherosclerotic cardiovascular disease than conventional antihypertensive agents. Moreover, given that blockade of the renin-angiotensin system can inhibit renal sodium reabsorption and attenuate the fluid retention and edema associated with peripheral vasodilators,[27] such molecules could also lead to the development of new antidiabetic PPARγ ligands with improved safety profiles.

Herein we report that the biphenyl, nontetrazole Ang II receptor blocker (ARB) telmisartan can act as a partial agonist of PPARγ; influence the expression of PPARγ target genes involved in the regulation of carbohydrate and lipid metabolism; and reduce glucose, insulin, and triglyceride levels in an animal model of diet-induced insulin resistance. Molecular modeling studies suggest that telmisartan might influence PPARγ activity by interacting with regions of the ligand-binding domain (LBD) that are not typically engaged by full agonists of the receptor. In contrast, molecular modeling and receptor transactivation studies indicate that other ARBs lack telmisartan's potential for receptor interaction and have relatively little or no effect on PPARγ activity. These findings (1) demonstrate that at least one of the ARBs currently in clinical use has the capacity to activate PPARγ and (2) suggest a number of interesting opportunities for developing improved therapeutic approaches to the metabolic syndrome as well as type 2 diabetes and other clinical disorders that might be influenced by activity of the renin-angiotensin system, PPARγ, or both.

Methods

Experimental Compounds

Ang II receptor antagonists and thiazolidinedione ligands of PPARγ were obtained from the pharmacy and purified by high-performance liquid chromatography. Candesartan cilexetil and olmesartan medoxomil were hydrolyzed to their active forms before high-performance liquid chromatography purification. Both losartan and its more active metabolite EXP 3174 were used in some studies.

PPAR Transactivation Assays

PPARγ activity was determined by transactivation assays in CV-1 cells (CCL-70 line from the American Type Culture Collection [ATCC], Manassas, Va) transfected by use of the GenePorter transfection reagent (Gene Therapy Systems) delivering 200 ng of the murine PPARγ expression plasmid pGAL4-mPPARγ LBD, 1 μg luciferase reporter plasmid pUAS-tk-luc (both courteously provided by P. Tontonoz, Howard Hughes Medical Institute and Department of Pathology, University of California, Los Angeles), and 400 ng of pCMVSport β-gal (Gibco) as an internal control. Twenty-four hours after transfection, cells were treated with varying concentrations of the test compounds and incubated for an additional 24 hours. Cell extracts were assayed for luciferase and β-galactosidase activity with Promega assay systems. All treatments were performed in triplicate and normalized for β-galactosidase activity. Assays for PPARα and PPARδ activity were performed in a similar fashion with pGAL-mPPARα LBD and pGAL4-mPPARδ LBD plasmids courteously supplied by R. Evans, Salk Institute for Biological Studies and Howard Hughes Medical Center. Agonist concentrations yielding half-maximal activation (EC_{50} values) were calculated with GraphPad Prism version 3.03 software (GraphPad Software, Inc). In some experiments, a range of concentrations of the ARBs was combined with a fixed concentration of rosiglitazone to test for receptor antagonist activity. To determine ligand effects on the full-length human PPARγ receptor, we also used a cloned full-length human receptor (pDEST-hPPARγ) obtained from Dr N. Takahasi (Kyoto University, Kyoto, Japan). The reporter was p3xPPRE-tk-luc, and transfections were performed with 400 ng of each plasmid.

Molecular Modeling in PPARγ

Computational studies were performed on a Silicon Graphics Octane 2 workstation equipped with 2 parallel R12000 processors, V6 graphics board, and 512-MB memory. Energy minimization and molecular dynamics were accomplished in the DISCOVER module of InsightII (Accelrys Inc), whereas the AFFINITY module of InsightII was used for docking studies.[28] The crystal structure of the partial agonist GW0072 in the PPARγ LBD was used for telmisartan docking studies (PDB code 4PRG).[29] Hydrogen atoms were added, and the structure was subjected to preliminary minimization, followed by molecular dynamics to relieve internal strain while heavy atoms were tethered to their original positions. Docking was performed with a previously described protocol.[30]

Adipocyte Differentiation Assay

Differentiation assays were performed on murine 3T3-L1 preadipocytes (CCL-173 line from the ATCC) by a modification of the technique of Smith et al.[31] After the cells reached confluence, they were incubated in Dulbecco's modified Eagle medium containing 1.0 μmol/L dexamethasone, 5 μg /mL insulin, and 0.5 mmol/L 1-methyl-3-isobutylxanthine with 5% calf serum for 32 hours, after which the cells were washed with phosphate-buffered saline and incubated in medium containing varying concentrations of test compounds or the vehicle dimethyl sulfoxide (DMSO). Five days after treatment, cells were fixed and stained with oil red O. Quantitative evaluation of adipogenesis was performed with a modified version of the method of Ramirez-Zacarias et al[32] by measuring absorbance at 510 nm.

PPARγ Target Gene Expression Assays

Expression levels of the PPARγ target genes *AP2* and *CD36* were determined by real-time polymerase chain reaction (PCR) of cDNA prepared from 3T3-L1 preadipocytes incubated with the test compounds or DMSO vehicle control for 3 days. Additional studies were performed in adult human subcutaneous adipocytes (Cambrex, Walkersville, Md) to determine the effects of the test compounds on expression of *PCK1* that codes for phosphoenolpyruvate carboxykinase-1 (PEPCK-C). This gene was selected because PEPCK-C has been proposed to be a key mediator of the effects of PPARγ ligands on fatty acid metabolism and insulin sensitivity.[33] Previous studies have also shown that acetyl coenzyme A carboxylase (ACC2) is a major regulator of muscle fatty acid metabolism.[34,35] Therefore, we also tested the effects of rosiglitazone, telmisartan, irbesartan, and valsartan on the expression of ACC2 in murine muscle myotubes that had been derived by differentiation of the C2C12 myoblast cell line (CRL 1772 from the ATCC). Total RNA was isolated by standard methods, and cDNA was prepared and analyzed by real-time PCR testing with SYBR green reagents, as previously described.[36] The cyclophilin (peptidylprolyl isomerase A) gene was used as an internal control, with results being determined in triplicate by the preferred method of Muller et al[37,38] and displayed as the amount of mRNA in drug-treated samples relative to that in the vehicle-treated control, which was arbitrarily defined as 1. Primer specificity was confirmed by melting point analysis for each primer pair. Primer sequences are available on request.

Dietary Model of Insulin Resistance

Male Sprague-Dawley rats were placed on a high-fat, high-carbohydrate diet (Teklad Diet TD03203 containing 60% fructose, 10% lard, and 0.06% magnesium) at 6 weeks of age. Two days after starting the diet, the rats were randomized into 3 different groups

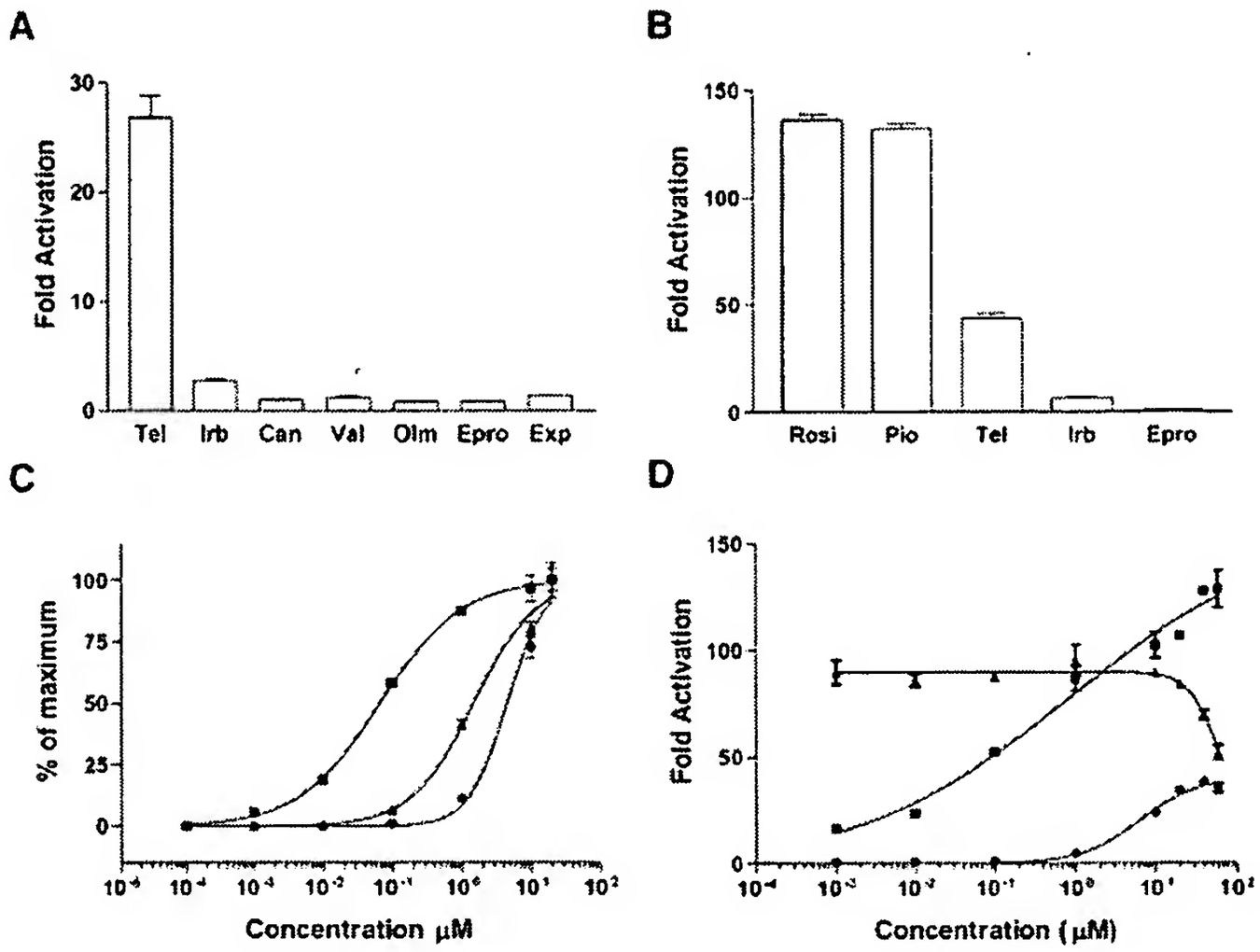

Figure 1. Telmisartan is a partial agonist of PPARγ. A, Comparison of the ability of different ARBs to activate PPARγ in a cell-based transient transfection assay. Cells were treated with 10 μmol/L telmisartan (Tel), irbesartan (Irb), candesartan (Can), valsartan (Val), olmesartan (Olm), eprosartan (Epro), or the EXP 3174 (Exp) active metabolite of losartan (Los). B, Comparison of the ability of different thiazolidinediones and ARBs to maximally activate PPARγ. Cells were treated with 40 μmol/L rosiglitazone (Rosi), pioglitazone (Pio), telmisartan (Tel), irbesartan (Irb), or eprosartan (Epro). C, Dose-response curves comparing potency of Rosi (squares), Pio (triangles), and Tel (circles) in the transient transfection assay. D, Mixing experiment showing that high concentrations of Tel can antagonize the ability of Rosi to activate PPARγ. Cells were treated with different concentrations of Rosi alone (squares), Tel alone (circles), or a mixture of 1 μmol/L Rosi with different concentrations of Tel (triangles).

(n=10 rats per group): group 1, telmisartan dose ≈5 mg/kg body weight per day; group 2, losartan dose ≈5 mg/kg body weight per day; and group 3, controls (no drug). The drugs were administered by dissolving the commercially available medications in the drinking water at an initial concentration of 40 mg/L. Because fluid intake did not scale linearly with body weight, the drug intakes based on body weight tended to decline over time. Therefore, drug concentrations in the drinking water were increased during the course of the study to help maintain the scheduled dosing. Food and fluid intakes were measured each day, and a pair-feeding protocol was followed to ensure equivalent food intakes among the 3 groups. Rats in the losartan group and the control group were pair-fed the same amount of chow consumed by the telmisartan group the day before. This ensured that the telmisartan group consumed at least as much if not slightly more food than the other groups. The fluid intakes and therefore drug intakes were similar in the telmisartan and losartan groups. After 5 weeks, serum levels of glucose, insulin, and triglycerides were obtained in the semifasting state (the night before blood sampling the animals were given a restricted amount of chow equivalent to 3 g/100 g body weight at 5 PM, and blood was drawn the following morning through the tail vein in the unanesthetized state). The protocol was continued for an additional 9 weeks, at which time glucose tolerance testing (oral glucose tolerance test) was performed in conscious animals in the semifasted state by sampling blood for glucose and insulin measurements after oral administration of glucose, 100 mg/100 g body weight. Serum levels of glucose and triglycerides were measured by spectrophotometric methods, and insulin levels were measured by radioimmunoassay (Linco).

Statistical Analysis

Statistical analysis was performed by Student *t* test or ANOVA followed by Dunnett multiple comparison test or the Student-Newman-Keuls test for comparisons across multiple groups. Statistical significance was defined as $P<0.05$. Data are expressed as mean±SEM.

Results

Telmisartan Is a Partial Agonist of PPARγ

We tested the ability of different ARBs to activate PPARγ in a heterologous transactivation assay that eliminates interference from endogenous nuclear receptors (Figure 1A).[9] Thus, exogenously added drugs including rosiglitazone and telmisartan do not cause any activation of the reporter gene in the absence of the PPAR construct (data not shown). In the full assay system including the PPARγ construct, we found that telmisartan was the only ARB that caused substantial activation of PPARγ (Figure 1A). Although irbesartan appeared to cause slight activation of PPARγ (2- to 3-fold activation) when tested at 10 μmol/L, none of the other ARBs, including the active metabolite of losartan, increased PPARγ activity when tested at this concentration (Figure 1A). Moreover, telmisartan was the only ARB that activated PPARγ when tested at lower concentrations (1 to 5 μmol/L) that can be achieved in plasma with conventional oral dosing (data not shown).[39] Telmisartan concentrations of <10 μmol/L did not significantly affect the activity of PPARα or PPARδ, although 25 μmol/L telmisartan appeared to cause a modest activation (4-fold) of PPARα (data not shown).

Telmisartan functioned as a moderately potent (EC_{50}=4.5 μmol/L), selective PPARγ partial agonist, activating the receptor to 25% to 30% of the maximum level achieved by the full agonists pioglitazone and rosiglitazone (Figure 1B and 1C). Corresponding EC_{50} values for rosiglitazone and pioglitazone were 0.066 and 1.5 μmol/L, respectively. As expected for a partial agonist, high concentrations of telmisartan (>10 to 20 μmol/L) in the presence of the full agonist rosiglitazone (1 μmol/L) attenuated the level of receptor activation otherwise achieved by the full agonist alone (Figure 1D). It should be noted that under normal circumstances, plasma concentrations of telmisartan do not reach 10 to 20 μmol/L. Thus, in patients with diabetes, administration of telmisartan is unlikely to interfere with the therapeutic effects of a simultaneously administered thiazolidinedione. We also tested telmisartan for its ability to activate full-length human PPARγ by using the full-length receptor and the luciferase reporter gene fused to the tandemly repeated PPARγ DNA response element. At 10 and 1 μmol/L, rosiglitazone achieved activation levels of 16-fold, whereas the values for telmisartan were 6-fold and 3-fold, respectively. Other sartans were inactive up to 40 μmol/L.

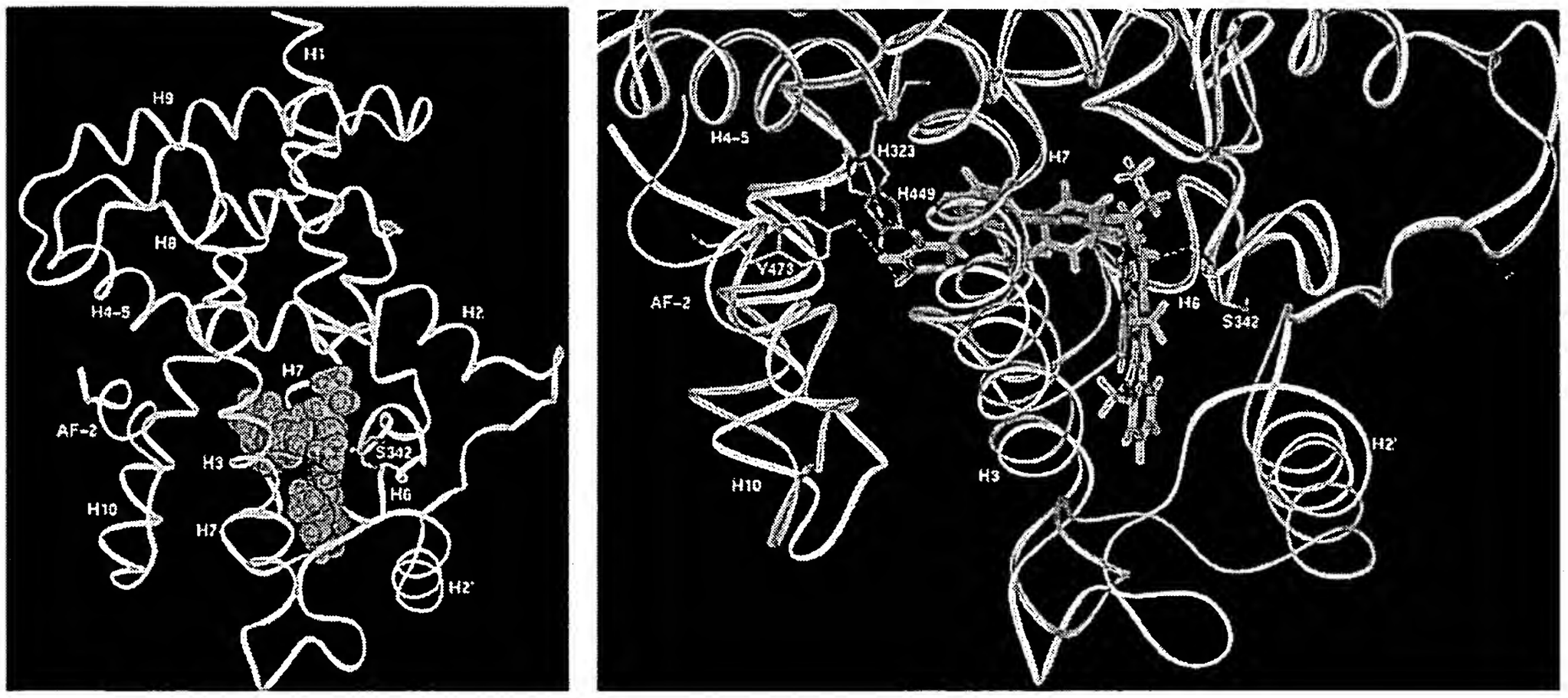

Figure 2. Binding mode of telmisartan in the PPARγ LBD. A, The protein backbone is illustrated as a yellow ribbon with telmisartan shown as a Van der Waals space–filling representation in blue. Ser342 is colored by atom types. B, Superimposition of telmisartan bound to the PPARγ LBD (extracted from PDB structure 4PRG) on the cocrystal structure of rosiglitazone and the PPARγ LBD (PDB structure 2PRG). The protein bound to telmisartan is shown as a yellow ribbon; the protein bound to rosiglitazone is shown as a magenta ribbon. Telmisartan and rosiglitazone are represented as sticks in blue and orange, respectively. Residues His323, His449, and Tyr473 of the rosiglitazone-bound protein and Ser342 of the telmisartan-bound protein are shown as sticks colored by atom types. Hydrogen bonding interactions are shown as dashed white lines.

Molecular Modeling of Telmisartan in the PPARγ LBD

Docking studies of the molecular binding mode in PPARγ revealed that telmisartan is surrounded by helixes H3, H6, and H7 (Figure 2A). The region of the LBD occupied by telmisartan is similar to that occupied by other partial agonists of PPARγ, including GW0072 and nTZDpa (data not shown).[29,40] The interaction of telmisartan with PPARγ might be explained by strong hydrophobic interactions with many of the residues forming the H3 and H7 helixes. Additionally, the hydrogen bond between the 1′-benzimidazole nitrogen and the amide proton of Ser342 might also contribute toward stabilization of this interaction (Figure 2A), particularly given that the partial agonists GW0072 and nTZDpa also form hydrogen bonds with the same serine residue.

The superimposition of telmisartan bound to PPARγ on the cocrystal structure of rosiglitazone and PPARγ is shown in Figure 2B. In contrast to the binding of full agonists such as rosiglitazone,[41] telmisartan, like other partial agonists including GW0072 and nTZDpa, does not appear to make direct contact with the activation function helix (AF-2) or with the adjacent histidine residues (Figure 2B). Interaction with the AF-2 helix has been shown to be responsible for receptor stabilization and activation by full agonists of PPARγ.[9,41] The lack of interaction of telmisartan with the AF-2 helix likely explains its inability to fully activate the receptor.

To further understand the structural changes in the protein induced by binding of ligands with different biologic profiles (full agonists vs partial agonists), we analyzed the crystal structures of the PPARγ LBD in its native form (PDB code 1PRG), the PPARγ LBD bound to the full agonist rosiglitazone (PDB code 2PRG), and the PPARγ LBD bound to the partial agonist GW0072 (PDB code 4PRG). As previously noted, the crystallographically determined structure of PPARγ bound to a partial agonist (GW0072) was more similar to the native (apo) structure than the crystallographically determined conformation of the protein bound to the full agonist rosiglitazone.[29] This was evidenced by the root mean square deviation (RMSD) of the backbone atoms that, for the partial agonist versus the apo protein, was 0.87, whereas this difference for the full agonist was 2.58. It is interesting to note that the shift in the structure of the native receptor induced by binding of GW0072 appears primarily to be a result of changes in the backbone conformation of helixes H3 and H7. The RMSD for the backbone atoms of helixes H3 and H7 in the 2 structures was found to be 0.79. Although binding of the full agonist resulted in a significant structural change in the native protein (RMSD 2.60 for the backbone atoms), the conformation of helixes H3 and H7 in this case did not differ significantly from the native protein (RMSD 0.44 for the backbone atoms). Based on these comparisons and the observation that protein-bound complexes of partial agonists like telmisartan, nTZDpa, and GW0072 are mainly stabilized by hydrophobic interactions with helixes H3 and H7, an alteration in the conformation of these helixes induced by partial agonists might contribute to the differences in receptor activation and target gene expression caused by such ligands compared with full agonists. In preliminary studies, high concentrations of telmisartan did not displace radiolabeled rosiglitazone from a recombinant form of the LBD (data not shown), further suggesting that telmisartan does not

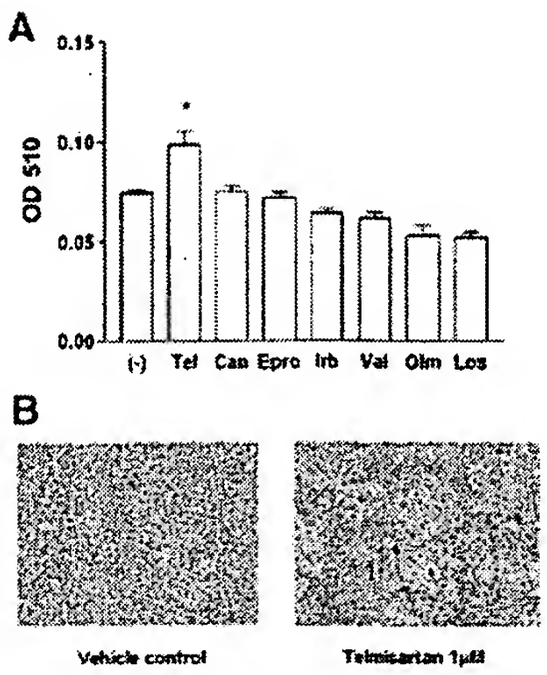

Figure 3. Telmisartan induces adipocyte differentiation in 3T3-L1 cells. A, Comparison of the effects of different ARBs on adipogenesis and lipid accumulation in 3T3-L1 cells as measured by the absorbance at 510 nm of oil red O eluted from stained cells. Cells were treated with 5 μmol/L of the indicated compounds or DMSO vehicle (−) for 5 days. *$P<0.01$ compared with all other groups by ANOVA and Dunnett multiple comparison test. Drug abbreviations are as in the legend to Figure 1. B, Oil red O staining of 3T3-L1 cells treated with 1 μmol/L telmisartan or DMSO vehicle control for 5 days.

interact with the receptor in the same fashion as conventional agonists of PPARγ.

Docking studies of several tetrazole-containing ARBs, including losartan, EXP 3174, irbesartan, olmesartan, and candesartan, in the PPARγ LBD revealed a different binding mode than for telmisartan. These tetrazole-containing ARBs made contacts with residues of helix H3 but not H7. Eprosartan, a nontetrazole ARB, was seen to interact with residues of helix H7 but not H3. None of these ARBs revealed a hydrogen bond with the binding domain or any interaction with the AF-2 helix. Thus, insufficient interaction with the PPARγ LBD might explain the inability or poor ability of these other ARBs to activate the protein.

Telmisartan Induces Adipocyte Differentiation

PPARγ plays a critical role in adipogenesis and has been shown to be necessary and sufficient for fat cell differentiation in cultured cells and in mice.[8,42] Therefore, we compared the ability of telmisartan and other ARBs to induce adipocyte differentiation of 3T3-L1 cells, a well-known characteristic of PPARγ ligands.[7,9] Telmisartan but none of the other ARBs clearly induced adipogenesis when tested at a concentration of 1 to 5 μmol/L (Figure 3 and data not shown). Although a higher concentration of irbesartan (10 μmol/L) induced adipocyte differentiation, none of the other ARBs, including the active metabolite of losartan, induced adipogenesis even when tested at concentrations of up to 25 μmol/L. In other experiments (data not shown), we found that the level of adipogenesis induced by rosiglitazone was ≈2 to 3 times greater than that induced by telmisartan. The relatively modest effect of telmisartan on adipogenesis is not surprising, given that other partial agonists of PPARγ have also been found to be relatively weak stimulators, or even inhibitors, of adipogenesis compared with full agonists such as rosiglitazone.[29,40,43]

Telmisartan Selectively Modulates the Expression of PPARγ Target Genes

PPARγ ligands influence the expression of multiple genes in differentiating preadipocytes and in mature adipocytes, such as *AP2* (*FABP4*) and *CD36*.[44–46] When tested at a concentration of 5 μmol/L, only telmisartan and irbesartan caused a substantial (>2-fold) increase in the expression of *AP2* and *CD36* in 3T3-L1 preadipocytes (Figure 4A and 4B). Whereas telmisartan and irbesartan both increased the expression of *AP2* and *CD36*, they appeared to have different effects on the expression of 2 other important genes related to lipid metabolism. In differentiated human adipocytes, telmisartan was the only ARB at concentrations of 2.5 to 5.0 μmol/L that clearly increased the expression of the *PCK1* gene encoding

Figure 4. Telmisartan influences the expression of PPARγ target genes in differentiating 3T3-L1 cells, mature human adipocytes, and murine myotubes, as measured by real-time PCR. A, Comparison of the effects of different ARBs on *AP2* expression in 3T3-L1 cells. B, Comparison of the effects of different ARBs on *CD36* expression in 3T3-L1 cells. C, Comparison of the effects of different ARBs on expression of the *PCK1* target gene encoding PEPCK in mature human subcutaneous adipocytes. D, Comparison of the effects of telmisartan, rosiglitazone, irbesartan, and valsartan on *ACC2* expression in differentiated C2C12 murine myotubes. For studies of *AP2*, *CD36*, and *ACC2*, cells were treated with 5 μmol/L of the indicated compounds or DMSO vehicle control for 3 days. For studies of *PCK1*, human adipocytes were treated with 2.5 μmol/L of the indicated compounds or DMSO vehicle control for 3 days. The real-time PCR assays were performed in triplicate, and in all panels each value represents the amount of mRNA in drug-treated samples relative to that in the vehicle-treated control, which was arbitrarily defined as 1. Drug abbreviations are as in the legend to Figure 1.

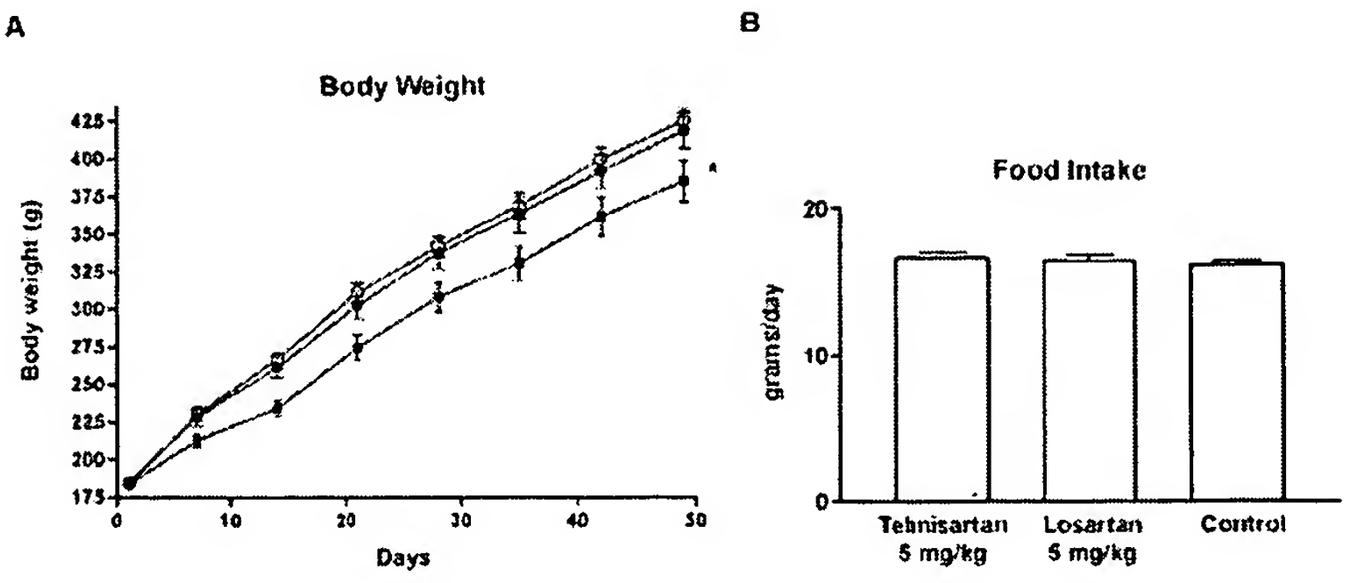

Figure 5. Telmisartan attenuates weight gain in rats fed a high-fat, high-carbohydrate diet. A, Mean body weights in rats given telmisartan, 5 mg/kg body weight per day (solid squares); losartan, 5 mg/kg body weight per day (solid circles); and in controls not given any drugs (open circles). B, Mean daily food intakes in rats treated with telmisartan or losartan and in untreated controls. Drug treatment groups and initial daily drug doses are listed below each bar graph. *$P<0.01$ for effects of drug, time, and drug-time interaction on body weight by 2-way ANOVA.

PEPCK-C (Figure 4C). This is noteworthy because the induction of PEPCK-C activity in adipocytes and the resultant increases in glyceroneogenesis and fatty acid reesterification have been proposed to play an essential role in the antidiabetic actions of PPARγ ligands.[33] In murine muscle myotubes, 5.0 μmol/L telmisartan caused a 60% to 70% decrease in the expression of *ACC2*, whereas rosiglitazone, irbesartan, and valsartan had little or no effect on *ACC2* expression (Figure 4D). ACC2 is a major regulator of muscle fatty acid metabolism, because this enzyme generates malonyl coenzyme A, a potent inhibitor of muscle carnitine palmitoyltransferase-1, which is a critical enzyme involved in fatty acid uptake in mitochondria. Accordingly, decreases in *ACC2* expression might be expected to promote increased fatty acid oxidation in skeletal muscle.[35]

Telmisartan Improves Glucose, Insulin, and Triglyceride Levels

Figure 5A shows body weights in control rats and in rats given either telmisartan or losartan. The 2-way ANOVA showed a significant effect of the drug ($P<0.01$), time ($P<0.01$), and a drug–time interaction ($P<0.01$) on body weight. Telmisartan administration caused a significant attenuation of weight gain compared with losartan and control groups (≈10%), whereas losartan appeared to have little or no effect on body weight compared with controls. Remarkably, the telmisartan-induced attenuation of weight gain could not be attributed to reduced energy intake, because daily food consumption was nearly identical in all groups as a result of the pair-feeding protocol (Figure 5B). Fluid intakes were similar among all 3 groups (data not shown).

Serum glucose levels measured after 5 weeks of treatment were significantly decreased in the telmisartan group compared with both the losartan group ($P<0.01$) and the control group ($P<0.001$) by ANOVA and Student-Newman-Keuls testing (Figure 6). Serum insulin levels also tended to be lower in the telmisartan-treated animals (Figure 6). Although overall ANOVA testing did not achieve statistical significance ($P=0.09$), the results of individual comparisons were consistent with lower insulin levels in the telmisartan group compared with the losartan group and the control group (both $P=0.025$ by 1-tailed t testing and $P<0.10$ by Student-Newman-Keuls testing; Figure 6B). Serum triglycerides were significantly decreased in the telmisartan treated group compared with both the losartan group ($P<0.05$) and the control group ($P<0.01$) by ANOVA and Student-Newman-Keuls testing; Figure 6).

During the oral glucose tolerance test, serum levels of glucose were similar among all 3 groups (Figure 7). However, serum insulin levels were significantly lower in the telmisartan group compared with both the losartan group and control group throughout the glucose tolerance test (Figure 7). The area under the curve for insulin in the telmisartan-treated rats, 3.7 ± 0.3 ng · mL^{-1} · 2 h^{-1}, was significantly lower than in the losartan-treated rats (6.0 ± 0.7 ng · mL^{-1} · 2 h^{-1}) and the control rats (5.3 ± 0.7 ng · mL^{-1} · 2 h^{-1}; $P<0.05$ by ANOVA and Student-Newman-Keuls testing). Compared with controls, losartan did not have significant effects on any of the parameters measured.

Discussion

On the basis of cellular assays of PPARγ activation, we have found that the Ang II receptor antagonist telmisartan is also a partial agonist of PPARγ, a well-known target of insulin-sensitizing drugs used to treat type 2 diabetes. In contrast, none of the other ARBs affected PPARγ activity with the possible exception of irbesartan, which appeared to cause a modest activation of the receptor when tested at a concentration of 10 μmol/L. In addition to activating PPARγ in cell-based transactivation assays, telmisartan increased the expression of known PPARγ target genes in both murine preadipocyte fibroblasts and human subcutaneous adipocytes and induced adipogenesis in 3T3-L1 preadipocyte fibroblasts, as expected for a PPARγ activator. Finally, in rats fed a high-fat, high-carbohydrate diet, orally administered telmisartan reduced glucose, insulin, and triglyceride levels, whereas losartan did not. In preliminary studies in obese Zucker rats that harbor mutant leptin receptors (data not shown), telmisartan did not appear to affect glucose, insulin, or triglyceride levels, suggesting that at least some of the beneficial metabolic effects of telmisartan might depend on the presence of an intact leptin signaling system.

The mechanism whereby telmisartan activates PPARγ remains to be determined; however, given the substantial chemical structural differences between telmisartan and all of the other commercially available ARBs, it is not surprising that telmisartan has unique biologic properties. There are a number of possible mechanisms that could theoretically mediate the effects of telmisartan on PPARγ activity, including but not limited to effects on the conformation or phos-

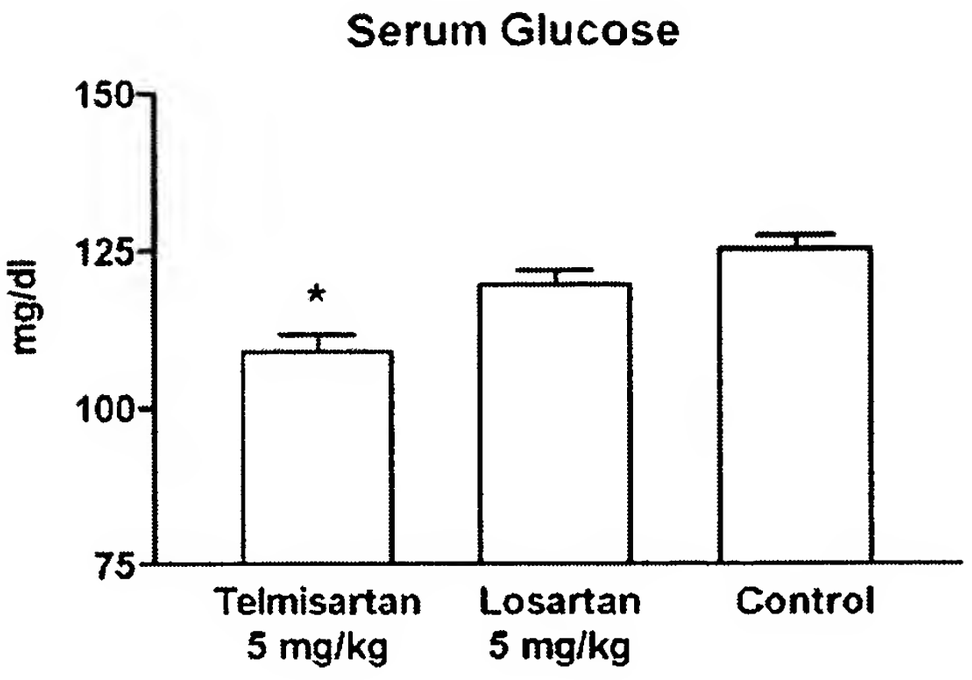

Figure 6. Telmisartan reduces serum levels of glucose, insulin, and triglycerides. Drug treatment groups and initial daily drug doses are listed below each bar graph. *$P<0.001$ by ANOVA, $P<0.001$ compared with control group, and $P<0.01$ compared with losartan group by Student-Newman-Keuls testing. **$P=0.09$ by ANOVA, $P=0.025$ compared with control group and losartan group by 1-tailed t testing, and $P=0.09$ compared with control group and losartan group by Student-Newman-Keuls testing. ***$P<0.01$ by ANOVA, $P<0.01$ compared with control group, and $P<0.05$ compared with losartan group by Student-Newman-Keuls testing.

phorylation status of the receptor, effects on the activity of coactivators or corepressors that modulate the transcriptional effects of PPARγ, or even effects on endogenous ligands of PPARγ. For example, molecular modeling studies suggest that telmisartan might fit within the LBD of PPARγ in a complex that is stabilized by hydrophobic interactions with helixes H3 and H7, as well as by a hydrogen bond with the amide proton of Ser342. This binding mode is similar to that observed for the partial agonist GW0072.[29] In contrast, the other ARBs do not appear to have the same potential for interaction with the receptor like telmisartan. Although it remains to be determined whether the unusual ability of telmisartan to activate PPARγ is indeed related to the receptor interactions identified in the modeling studies, it is clear that substantial differences exist between the chemical structures of telmisartan and the other ARBs.

Although telmisartan was developed with the goal of selectively blocking the Ang II type 1 receptor to treat hypertension, the finding that this molecule can also activate PPARγ has potentially important therapeutic implications for pharmacological treatment of the metabolic syndrome, type 2 diabetes, and other clinical disorders that might be responsive to PPARγ activators.[47] Because hypertension frequently occurs together with insulin resistance and dyslipidemia,[48] the availability of multifunctional molecules that treat more than just increased blood pressure or the associated metabolic disturbances could be of considerable clinical value. It is widely believed that the currently available ARBs are metabolically neutral and have little or no impact on carbohydrate and lipid metabolism when administered in conventional doses used to treat hypertension.[26] However, the current findings suggest that telmisartan might be an exception in this regard and provide insight into new strategies for developing molecules that could improve many if not all of the biochemical and blood pressure disturbances that compose the metabolic syndrome.[47]

It should be emphasized that telmisartan is a partial agonist of PPARγ and appears to function as a selective PPAR modulator with different effects on gene expression than a full agonist of PPARγ like rosiglitazone. It is well known that partial agonists of PPARγ can exert different effects on gene expression patterns than do full agonists and that even full agonists might differ among themselves with respect to their precise effects on gene expression profiles.[40,49] Thus, it is not surprising that telmisartan, but not rosiglitazone, affected the expression of *ACC2*, a key gene involved in the regulation of muscle fatty acid metabolism. In fact, intense interest exists in the development of selective PPARγ modulators that can exert beneficial effects on the expression of genes that regulate carbohydrate and lipid metabolism without causing changes in gene expression that promote weight gain, fluid retention, or the other adverse effects associated with administration of conventional PPARγ activators.[29,40,43,50] In the current study, it is noteworthy that telmisartan attenuated weight gain despite the use of a pair-feeding protocol that ensured comparable food intakes among all of the experimental groups. Other partial agonists of PPARγ have also been shown to attenuate the weight gain ordinarily induced by a high-fat diet.[40,49] The current observations raise the intriguing possibility that telmisartan might have the capacity to influence genes that regulate energy metabolism in vivo and should motivate future studies on the ability of telmisartan to attenuate weight gain in humans consuming high-fat, high-carbohydrate diets.

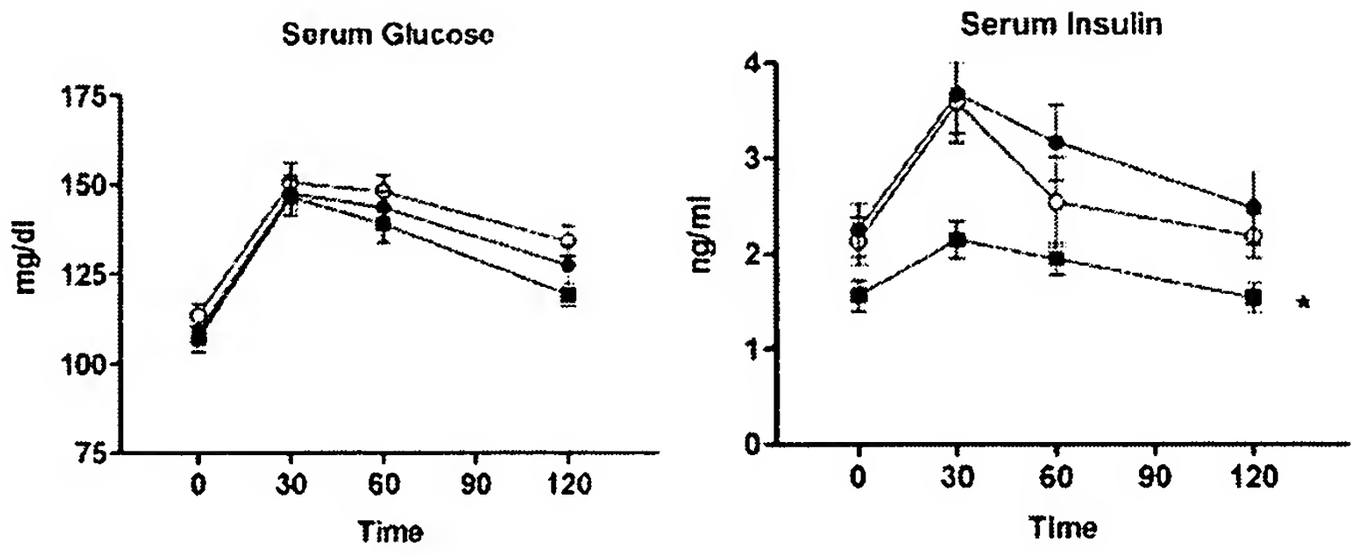

Figure 7. Telmisartan reduces insulin levels during oral glucose tolerance testing in rats fed a high-fat, high-carbohydrate diet. Serum glucose and insulin levels after oral administration of glucose, 100 mg/100 g body weight, to rats treated with telmisartan (solid squares), losartan (solid circles), or controls (open circles). *Area under the curve for insulin in the telmisartan-treated rats, 3.7±0.3 $ng \cdot mL^{-1} \cdot 2\ h^{-1}$, was significantly lower than in the losartan-treated rats (6.0±0.7 3 $ng \cdot mL^{-1}$) and control rats (5.3±0.7 3 $ng \cdot mL^{-1} \cdot 2\ h^{-1}$; $P<0.05$ by ANOVA and Student Newman-Keuls testing).

In light of a number of clinical and experimental studies suggesting that angiotensin-converting enzyme (ACE) inhibitors can improve insulin sensitivity and decrease the incidence of new-onset type 2 diabetes in patients with hypertension,[51–54] the question arises as to whether pharmacologic interruption of the renin-angiotensin system per se should be expected to lead to improvements in carbohydrate and lipid metabolism. However, recent studies have indicated that the insulin-sensitizing effects of ACE inhibitors might be more closely related to their effects on kinin metabolism rather than their effects on the renin-angiotensin system.[54–56] Thus, based on the results of studies with ACE inhibitors, it could not have been predicted that any of the existing ARBs would activate PPARγ and improve the disturbances in carbohydrate and lipid metabolism that are characteristic of the metabolic syndrome.

Although it is generally believed that ARBs do not exert significant effects on carbohydrate and lipid metabolism, such views are based largely on the results of clinical trials and experimental studies that have been conducted with ARBs that are structurally quite different from telmisartan. For example, measurements of systemic insulin action with the euglycemic clamp technique have failed to reveal any consistent effect of losartan on insulin sensitivity.[54] In the recent LIFE trial, the incidence of new-onset type 2 diabetes was reported to be significantly lower in hypertensive subjects treated with losartan than in those treated with atenolol, suggesting potential antidiabetic effects of angiotensin receptor blockade.[57] However, given the known diabetogenic effects of β-adrenergic blockers, it is possible that the lower incidence of new-onset diabetes in the losartan arm of the trial was related to a prodiabetic effect of atenolol rather than an antidiabetic effect of ARB. In the CHARM Preserved trial, the incidence of new-onset type 2 diabetes was significantly lower in subjects given candesartan than in those given placebo.[58] However, in other trials including CHARM Added, CHARM Alternative, and SCOPE, there was no significant difference in the incidence of new-onset diabetes in subjects given candesartan compared with controls.[59–61] In the ALPINE and CROSS studies, candesartan appeared to have little or no effect on serum levels of insulin, glucose, or triglycerides.[62,63] Although candesartan administration appeared to improve an indirect estimate of insulin action in the CROSS study, it failed to show any effect on the HOMA (homeostasis model assessment) index of insulin resistance in the ALPINE study.[62,63] Finally, in the obese Zucker rat, Henriksen et al[64] found that oral administration of an extremely high dose of irbesartan (50 mg/kg) improved insulin sensitivity but apparently failed to improve lipid levels. Thus, although the results of Henriksen et al are consistent with our finding of a weak effect of high concentrations of irbesartan on PPARγ activity, they do not imply that conventional doses of irbesartan or any other ARB could be used to activate PPARγ in vivo or treat the metabolic syndrome.

Recently, Janke and colleagues[65] have reported that very high concentrations of Ang II can inhibit differentiation of human preadipocytes and that high concentrations of irbesartan can enhance adipogenesis. On the basis of these findings and recent evidence showing that a lack of adipose tissue can promote diabetes by causing excess storage of fat in muscle, liver, and pancreas,[66] Sharma and colleagues[67] have proposed that blockade of the renin-angiotensin system per se might prevent diabetes by promoting the recruitment and differentiation of adipocytes. In the current studies, we found that moderate concentrations of telmisartan and high concentrations of irbesartan could activate PPARγ and promote adipogenesis; however, other ARBs failed to show any effects on PPARγ activity or adipogenesis. PPARγ is known to play a pivotal role in adipogenesis, whereas the effect of the renin-angiotensin system on fat cell differentiation is much less clear, particularly given that very high concentrations of Ang II are typically required to observe effects on adipogenesis. If the adipogenic effects of telmisartan and irbesartan were related to blockade of the renin-angiotensin system in the cells we tested, one would have expected other ARBs also to induce adipogenesis. Moreover, the concentrations of telmisartan and irbesartan required to induce adipogenesis are far greater than the concentrations required to block the Ang II type 1 receptor, further suggesting that the in vitro effects of telmisartan or other ARBs on adipogenesis in the cells that we tested are unlikely to be related to Ang II receptor blockade. Thus, although our findings are consistent with the results of Sharma and colleagues, we believe that at least in the cell line we studied, the effects of telmisartan and

irbesartan on adipocyte differentiation are more likely related to their ability to activate PPARγ rather than their ability to block Ang II receptors.

Perspectives

Aside from the potential use of telmisartan for the prevention and treatment of diabetes and the metabolic syndrome, the discovery that telmisartan can activate PPARγ has a number of implications for the development of next-generation molecules for treating clinical disorders that are influenced by activity of the renin-angiotensin system and PPARγ. Notwithstanding the results of the recent ALLHAT trial,[53] drugs that interrupt the renin-angiotensin system are considered by many to be superior for preventing hypertension-related target-organ damage than are antihypertensive agents that were not designed to interrupt the renin-angiotensin system.[68–70] The development of novel ARBs that ameliorate insulin resistance and dyslipidemia as well as hypertension could provide even more effective options for preventing target-organ damage and cardiovascular disease in patients with hypertension, diabetes, or both. Such agents, either alone or in combination with ACE inhibitors, could also be useful for the prevention of new-onset diabetes in patients with hypertension or in other high-risk populations. Finally, given the known inhibitory effects of Ang II receptor blockade on renal sodium reabsorption, the current findings could provide new opportunities for developing antidiabetic PPARγ ligands without the adverse effects of fluid retention, peripheral edema, and heart failure associated with conventional agonists of PPARγ, like rosiglitazone and pioglitazone.[20–22]

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References

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature.* 2001;414:782–787.
2. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications, part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med.* 1998;15: 539–553.
3. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA.* 2001;285:2486–2497.
4. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA.* 2002;288:2709–2716.
5. Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988–1994. *Arch Intern Med.* 2003;163:427–436.
6. Laaksonen DE, Lakka HM. Niskanen LK, Kaplan GA, Salonen JT, Lakka TA. Metabolic syndrome and development of diabetes mellitus: application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study. *Am J Epidemiol.* 2002;156:1070–1077.
7. Lehmann JM. Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptorγ (PPARγ). *J Biol Chem.* 1995; 270:12953–12956.
8. Rosen ED, Spiegelman BM. PPARγ: a nuclear regulator of metabolism, differentiation, and cell growth. *J Biol Chem.* 2001;276:37731–37734.
9. Willson TM, Brown PJ, Sternbach DD, Henke BR. The PPARs: from orphan receptors to drug discovery. *J Med Chem.* 2000;43:527–550.
10. Berger J, Moller DE. The mechanisms of action of PPARs. *Annu Rev Med.* 2002;53:409–435.
11. Picard F, Auwerx J. PPARγ and glucose homeostasis. *Annu Rev Nutr.* 2002;22:167–197.
12. Walczak R, Tontonoz P. PPARadigms and PPARadoxes: expanding roles for PPAR-γ in the control of lipid metabolism. *J Lipid Res.* 2002;43:177–186.
13. Wakino S, Law RE, Hsueh WA. Vascular protective effects by activation of nuclear receptor PPAR-γ. *J Diabetes Complicat.* 2002;16:46–49.
14. Hsueh WA, Law R. The central role of fat and effect of peroxisome proliferator-activated receptor-γ on progression of insulin resistance and cardiovascular disease. *Am J Cardiol.* 2003;92:3J–9J.
15. Schiffrin EL, Amiri F, Benkirane K, Iglarz M, Diep QN. Peroxisome proliferator-activated receptors: vascular and cardiac effects in hypertension. *Hypertension.* 2003;42:664–668.
16. Kotchen TA. Attenuation of hypertension by insulin-sensitizing agents. *Hypertension.* 1996;28:219–223.
17. Verma S, Bhanot S, Arikawa E, Yao L, McNeill JH. Direct vasodepressor effects of pioglitazone in spontaneously hypertensive rats. *Pharmacology.* 1998;56:7–16.
18. Home PD, Piaditis G, Koelendorf K, Raz I, Murphy L, Smith PL, Kler L, Dave J, Tandy D. Anti-hypertensive effect of farglitazar, a tyrosine based non-thiazolidinedione PPAR-γ agonist, in patients with type 2 diabetes and hypertension. *Diabetes.* 2001;50:A117.
19. Buchanan TA, Meehan WP, Jeng YY, Yang D, Chan TM, Nadler JL, Scott S, Rude RK, Hsueh WA. Blood pressure lowering by pioglitazone; evidence for a direct vascular effect. *J Clin Invest.* 1995;96:354–360.
20. Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, Le Winter M, Porte D, Semenkovich CF, Smith S, Young LH, Kahn R. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. *Diabetes Care.* 2004;27:256–263.
21. Delea T, Edelsberg J, Hagiwara M, Oster G, Phillips LS. Use of thiazolidinediones and risk of heart failure in people with type 2 diabetes: a retrospective cohort study. *Diabetes Care.* 2003;26:2983–2989.
22. Mudaliar S, Chang AR, Henry RR. Thiazolidinediones, peripheral edema, and type 2 diabetes: incidence, pathophysiology, and clinical implications. *Endocr Pract.* 2003;9:406–416.
23. Edsberg B, Strand J, Sandeman D, Skovsted B. Efficacy and safety of ragaglitazar, a novel dual PPARα and PPARγ agonist, in patients with type 2 diabetes. In: *Proceedings of the 38th European Association for the Study of Diabetes*; Budapest, Hungary; 2002. PS53.
24. Abou-Donia M, Fiedorek FT, Wilson GG, Frith L, Patel J. Monotherapy with GI262570, a non-thiazolidinedione PPARγ agonist, improves glycemic control in type 2 diabetes mellitus patients. In: *Proceedings of the 36th European Association for the Study of Diabetes.* Tel Aviv, Israel; 2000. Abstract 726.
25. Pershadsingh HA, Benson SC, Ho CI, Avery MA, Kurtz TW. Identification of PPAR-γ activators that do not promote fluid retention and edema: implications for treating insulin resistant hypertension and the metabolic syndrome. In: *Proceedings of the Endocrine Society Symposium on Nuclear Receptors in Cardiovascular Disease. Hot Topics in Endocrinology.* San Diego, Calif: 2003. Abstract 29.
26. Epstein M, Brunner HR, eds. *Angiotensin II Receptor Antagonists.* 1st ed. Philadelphia, Pa: Hanley and Belfus; 2001.
27. Messerli FH. Vasodilatory edema: a common side effect of antihypertensive therapy. *Am J Hypertens.* 2001;14:978–979.
28. Luty BA, Wassserman ZR, Stouten PFW, Hodge CN, Zacharias M, McCammon JA. A molecular mechanics/grid method for evaluation of ligand-receptor interactions. *J Comput Chem.* 1995;16:454–464.
29. Oberfield JL, Collins JL, Holmes CP, Goreham DM, Cooper JP, Cobb JE, Lenhard JM, Hull-Ryde EA, Mohr CP, Blanchard SG, Parks DJ, Moore LB, Lehmann JM, Plunket K, Miller AB, Milburn MV, Kliewer SA, Willson TM. A peroxisome proliferator-activated receptor-γ ligand inhibits adipocyte differentiation. *Proc Natl Acad Sci U S A.* 1999;96:6102–6106.
30. Sabnis YA, Desai PV, Rosenthal PJ, Avery MA. Probing the structure of falcipain-3, a cysteine protease from *Plasmodium falciparum*: comparative protein modeling and docking studies. *Prot Sci.* 2003;12:501–509.

31. Smith PJ, Wise LS, Berkowitz R, Wan C, Rubin CS. Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3–L1 adipocytes. *J Biol Chem.* 1988;263:9402–9408.
32. Ramirez-Zacarias JL, Castro-Munozledo F, Kuri-Harcuch W. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with oil red O. *Histochemistry.* 1992;97:493–497.
33. Tordjman J, Chauvet G, Quette J, Beale EG, Forest C, Antoine B. Thiazolidinediones block fatty acid release by inducing glyceroneogenesis in fat cells. *J Biol Chem.* 2003;278:18785–18790.
34. Abu-Elheiga L, Oh W, Kordari P, Wakil SJ. Acetyl-CoA carboxylase 2 mutant mice are protected against obesity and diabetes induced by high-fat/high-carbohydrate diets. *Proc Natl Acad Sci U S A.* 2003;100:10207–10212.
35. Ruderman N, Flier JS. Cell biology: chewing the fat–ACC and energy balance. *Science.* 2001;291:2558–2559.
36. Qi N, Kazdova L, Zidek V, Landa V, Kren V, Pershadsingh HA, Lezin ES, Abumrad NA, Pravenec M, Kurtz TW. Pharmacogenetic evidence that CD36 is a key determinant of the metabolic effects of pioglitazone. *J Biol Chem.* 2002;277:48501–48507.
37. Muller PY, Janovjak H, Miserez AR, Dobbie Z. Processing of gene expression data generated by quantitative real-time RT-PCR. *Biotechniques.* 2002;32:1372–1379.
38. Muller PY, Janovjak H, Miserez AR. Processing of gene expression data generated by quantitative real time RT-PCR. *Biotechniques.* 2002;33:514.
39. Stangier J, Su CA, Roth W. Pharmacokinetics of orally and intravenously administered telmisartan in healthy young and elderly volunteers and in hypertensive patients. *J Int Med Res.* 2000;28:149–167.
40. Berger JP, Petro AE, Macnaul KL, Kelly LJ, Zhang BB, Richards K, Elbrecht A, Johnson BA, Zhou G, Doebber TW, Biswas C, Parikh M, Sharma N, Tanen MR, Thompson GM, Ventre J, Adams AD, Mosley R, Surwit RS, Moller DE. Distinct properties and advantages of a novel peroxisome proliferator- activated protein-γ selective modulator. *Mol Endocrinol.* 2003; 17:662–676.
41. Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ. *Nature.* 1998;395:137–143.
42. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. *Genes Dev.* 2000;14:1293–1307.
43. Reginato MJ, Bailey ST, Krakow SL, Minami C, Ishii S, Tanaka H, Lazar MA. A potent antidiabetic thiazolidinedione with unique peroxisome proliferator-activated receptor-γ -activating properties. *J Biol Chem.* 1998; 273:32679–32684.
44. Smith U, Gogg S, Johansson A, Olausson T, Rotter V, Svalstedt B. Thiazolidinediones (PPAR-γ agonists) but not PPAR-α agonists increase IRS-2 gene expression in 3T3–L1 and human adipocytes. *FASEB J.* 2001;15: 215–220.
45. Gerhold DL, Liu F, Jiang G, Li Z, Xu J, Lu M, Sachs JR, Bagchi A, Fridman A, Holder DJ, Doebber TW, Berger J, Elbrecht A, Moller DE, Zhang BB. Gene expression profile of adipocyte differentiation and its regulation by peroxisome proliferator-activated receptor-γ agonists. *Endocrinology.* 2002; 143:2106–2118.
46. Albrektsen T, Frederiksen KS, Holmes WE, Boel E, Taylor K, Fleckner J. Novel genes regulated by the insulin sensitizer rosiglitazone during adipocyte differentiation. *Diabetes.* 2002;51:1042–1051.
47. Pershadsingh HA, Kurtz TW. Insulin-sensitizing effects of telmisartan: implications for treating insulin resistant-hypertension and cardiovascular disease. *Diabetes Care.* In press.
48. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities: the role of insulin resistance and the sympathoadrenal system. *N Engl J Med.* 1996;334:374–381.
49. Camp HS, Li O, Wise SC, Hong YH, Frankowski CL, Shen X, Vanbogelen R, Leff T. Differential activation of peroxisome proliferator-activated receptor-γ by troglitazone and rosiglitazone. *Diabetes.* 2000;49:539–547.
50. Rocchi S, Picard F, Vamecq J, Gelman L, Potier N, Zeyer D, Dubuquoy L, Bac P, Champy MF, Plunket KD, Leesnitzer LM, Blanchard SG, Desreumaux P, Moras D, Renaud JP, Auwerx J. A unique PPAR-γ ligand with potent insulin-sensitizing yet weak adipogenic activity. *Mol Cell.* 2001; 8:737–747.
51. Yusuf S, Gerstein H, Hoogwerf B, Pogue J, Bosch J, Wolffenbuttel BH, Zinman B. Ramipril and the development of diabetes. *JAMA.* 2001;286: 1882–1885.
52. Hansson L, Lindholm LH, Niskanen L, Lanke J, Hedner T, Niklason A, Luomanmaki K, Dahlof B, de Faire U, Morlin C, Karlberg BE, Wester PO, Bjorck JE. Effect of angiotensin-converting-enzyme inhibition compared with conventional therapy on cardiovascular morbidity and mortality in hypertension: the Captopril Prevention Project (CAPPP) randomised trial. *Lancet.* 1999;353:611–616.
53. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA.* 2002;288:2981–2997.
54. Bernobich E, de Angelis L, Lerin C, Bellini G. The role of the angiotensin system in cardiac glucose homeostasis: therapeutic implications. *Drugs.* 2002;62:1295–1314.
55. Tomiyama H, Kushiro T, Abeta H, Ishii T, Takahashi A, Furukawa L, Asagami T, Hino T, Saito F, Otsuka Y, et al. Kinins contribute to the improvement of insulin sensitivity during treatment with angiotensin converting enzyme inhibitor. *Hypertension.* 1994;23:450–455.
56. Shiuchi T, Cui TX, Wu L, Nakagami H, Takeda-Matsubara Y, Iwai M, Horiuchi M. ACE inhibitor improves insulin resistance in diabetic mouse via bradykinin and NO. *Hypertension.* 2002;40:329–334.
57. Lindholm LH, Ibsen H, Borch-Johnsen K, Olsen MH, Wachtell K, Dahlof B, Devereux RB, Beevers G, de Faire U, Fyhrquist F, Julius S, Kjeldsen SE, Kristianson K, Lederballe-Pedersen O, Nieminen MS, Omvik P, Oparil S, Wedel H, Aurup P, Edelman JM, Snapinn S. Risk of new-onset diabetes in the Losartan Intervention For Endpoint reduction in hypertension study. *J Hypertens.* 2002;20:1879–1886.
58. Yusuf S, Pfeffer MA, Swedberg K, Granger CB, Held P, McMurray JJ, Michelson EL, Olofsson B, Ostergren J. Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the CHARM-Preserved Trial. *Lancet.* 2003;362:777–781.
59. Granger CB, McMurray JJ, Yusuf S, Held P, Michelson EL, Olofsson B, Ostergren J, Pfeffer MA, Swedberg K. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. *Lancet.* 2003;362:772–776.
60. McMurray JJ, Ostergren J, Swedberg K, Granger CB, Held P, Michelson EL, Olofsson B, Yusuf S, Pfeffer MA. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function taking angiotensin-converting-enzyme inhibitors: the CHARM-Added trial. *Lancet.* 2003;362:767–771.
61. Lithell H, Hansson L, Skoog I, Elmfeldt D, Hofman A, Olofsson B, Trenkwalder P, Zanchetti A. The Study on Cognition and Prognosis in the Elderly (SCOPE): principal results of a randomized double-blind intervention trial. *J Hypertens.* 2003;21:875–886.
62. Grassi G, Seravalle G, Dell'Oro R, Trevano FQ, Bombelli M, Scopelliti F, Facchini A, Mancia G. Comparative effects of candesartan and hydrochlorothiazide on blood pressure, insulin sensitivity, and sympathetic drive in obese hypertensive individuals: results of the CROSS study. *J Hypertens.* 2003;21:1761–1769.
63. Lindholm LH, Persson M, Alaupovic P, Carlberg B, Svensson A, Samuelsson O. Metabolic outcome during 1 year in newly detected hypertensives: results of the Antihypertensive Treatment and Lipid Profile in a North of Sweden Efficacy Evaluation (ALPINE study). *J Hypertens.* 2003;21:1563–1574.
64. Henriksen EJ, Jacob S, Kinnick TR, Teachey MK, Krekler M. Selective angiotensin II receptor antagonism reduces insulin resistance in obese Zucker rats. *Hypertension.* 2001;38:884–890.
65. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Mature adipocytes inhibit in vitro differentiation of human preadipocytes via angiotensin type 1 receptors. *Diabetes.* 2002;51:1699–1707.
66. Danforth E Jr. Failure of adipocyte differentiation causes type II diabetes mellitus? *Nat Genet.* 2000;26:13.
67. Sharma AM, Janke J, Gorzelniak K, Engeli S, Luft FC. Angiotensin blockade prevents type 2 diabetes by formation of fat cells. *Hypertension.* 2002;40: 609–611.
68. Taal MW, Brenner BM. Renoprotective benefits of RAS inhibition: from ACEI to angiotensin II antagonists. *Kidney Int.* 2000:57:1803–1817.
69. Dahlof B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, Faire U, Fyhrquist F, Ibsen H, Kristiansson K, Lederballe-Pedersen O, Lindholm LH, Nieminen MS, Omvik P, Oparil S, Wedel H. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet.* 2002;359: 995–1003.
70. Wing LM, Reid CM, Ryan P, Beilin LJ, Brown MA, Jennings GL, Johnston CI, McNeil JJ, Macdonald GJ, Marley JE, Morgan TO, West MJ. A comparison of outcomes with angiotensin-converting–enzyme inhibitors and diuretics for hypertension in the elderly. *N Engl J Med.* 2003;348:583–592.